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THE REACTION OF PLANT PROTOPLASM

A. R. HAAS

The reaction of protoplasm is one of the most important factors of metabolism. It is not, however, the apparent reaction (or total acidity), as shown by titration, which is of chief importance, but the actual reaction, as shown by the gas chain or by indicators. The total acidity includes both undissociated and dissociated acid, while the actual reaction depends only upon the latter.

In the case of a buffer solution[1] the total acidity may be very high, while the actual acidity may be very low. The higher the total acidity in this case the more difficult it becomes to change the actual reaction by the addition of acid or alkali. This applies to protoplasm, which always has the properties of a buffer solution (since it contains carbonates, phosphates, proteins, etc.). Hence if a low actual acidity is advantageous to the protoplasm, a high total acidity may be favorable, in that it preserves this desirable actual acidity.

So far as the writer is aware, no determinations of the actual reaction of plant protoplasm have been made by means of the gas chain (except a single determination of pineapple juice made by REED[2]). Only a few determinations have ever been made[3] by means of indicators.

The writer has investigated the reaction of a number of plants, including some which seemed to be of special interest on account of their unusual acidity. The method employed was to crush the cells and to determine the acidity of the juice by means of the gas chain. The gas chain used was essentially the form described by HILDEBRAND.[4]

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[1] Cf. HÖBER, H., Physikalische Chemie der Zelle und der Gewebe (pp. 118, 169). Berlin. 1914.

[2] Unpublished results.

[3] Cf. FRIEDENTHAL, H., Zeit. Allg. Physiol. 1:56. 1902. It is not known whether in these experiments the proper precautions were taken to crush all the cells and to secure plant juices which had as high an acidity as the cell contents.

[4] HILDEBRAND, J. H., Jour. Amer. Chem. Soc. **35**:869. 1913.

The method of obtaining the juice is of considerable importance. It has been shown by MAMELI,[5] by MARIE and GATIN,[6] and by DIXON and ATKINS,[7] that when tissue is crushed the juice which is first expressed contains a much lower concentration of electrolytes than that which is obtained when greater pressure is applied so as to crush more of the cells. It is obvious that when pressure is first applied and sap is squeezed out through the intact plasma membrane, the electrolytes may largely be retained within the cell because they are not able to pass freely through the membrane. It is desirable, therefore, to grind the tissue and rupture all of the cells. This was accomplished by thoroughly grinding the tissue in a mortar. Only a little tissue was ground at a time, and the grinding was continued until microscopic examination showed that all the cells were ruptured.

TABLE I

Material	Actual acidity of CO_2-free undiluted juice as determined by the gas chain	Total acidity as determined by ordinary titration methods
Lemon (fruit)	0.006N	
Cranberries (fruit), fresh material, peeled and unpeeled	0.004N	0.9172N (ripe fruit) 0.3194N (overripe, 0.3493N soft fruit)
Grapefruit (fruit)	0.001N	0.1927N
Apple (fruit)	0.0004N	0.0711N
Rhubarb (leaf-stalk) basal part	0.0007N	
intermediate part	0.0005N	0.1578N
green part below leaf blade	0.00022N	0.1681N
Orange (fruit)	0.00016N	0.0941N
Pineapple (ripe fruit), juice standing 2 hours	0.00009N	
Pineapple (ripe fruit), fresh juice	0.000035N	0.1377N
Green pepper (fruit)	0.0000038N	
Eggplant (fruit)	0.000002N	

Before determining the acidity of the juice, the CO_2 was driven off by means of a current of hydrogen. The results of the determinations are given in table I. The results in all cases represent the average of two or more closely concordant determinations.

[5] MAMELI, E., Atti Ist. Bot. Univ. Pavia **12**:285. 1908.

[6] MARIE, C. H., and GATIN, C. L., Dèterminations cryoscopiques effectuèes sur des sucs vègètaux. 1912.

[7] DIXON, H. H., and ATKINS, W. R. G., Proc. Roy. Dublin Soc. **13**:422. 1913.

The figures of the table show that the actual acidity bears no fixed relation to the total acidity, and that great variations are to be found in different plants, as well as in different tissues of the same plant.

The figures for actual acidity are surprisingly high in the case of the lemon and of the cranberry, especially in view of the prevalent opinion that protoplasm demands a neutral or nearly neutral reaction for normal metabolism. It is of interest, therefore, to inquire whether the figures represent the actual acidity of the protoplasm. In the lemon the acid juice is contained in sacs, the walls of which are composed of living cells, while the cavity is produced by the disintegration of cells. REED[8] has shown that the living cells of the walls contain oxidases whose activity is promptly inhibited by the acid contained in the cavity of the sac. Since the oxidases are active in the living cells, it follows that the protoplasm is by no means as acid as the juice in the sacs, and hence the figures given in table I cannot apply to the protoplasm in the case of the lemon.

With the cranberry the case seems to be different. In this fruit there are no sacs such as are found in the lemon; the juice is contained entirely in the cells. It is important, therefore, to ascertain whether these cells are dead or alive. In order to test this, the outer colored layer of cells was removed and the following results were obtained on the colorless cells:

1. Eosin failed to penetrate unboiled peeled cranberries, but penetrated readily into the boiled peeled cranberries. The difficulty due to the precipitation of eosin in acid solutions was obviated by frequent renewal of the eosin solution.

2. Ripe cranberries can be peeled without staining the white tissue beneath, while this is not possible in overripe, soft cranberries.

3. Unboiled, peeled cranberries in a red watery extract of cranberry peeling were unstained after several hours, while the indicator readily penetrated the boiled peeled cranberries.

All these tests go to show that the living cells of the cranberry have an actual acidity which is extremely high. It is quite possible, however, that the acid sap is contained in vacuoles rather than imbibed in the protoplasm proper.

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[8] REED, G. B., BOT. GAZ. 57:528. 1914.

Summary

1. The actual acidity and the total acidity of a number of plant tissues were determined.
2. There is no constant relation between the two, but great variations occur in different plants and in different parts of the same plant.
3. In one case (cranberry fruits) the surprisingly high actual acidity of 0.004N (as determined by the gas chain) was found in the living cells.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY